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# A STUDY OF THE NATURE OF STREPTOLYSIN.*

## SECOND CONTRIBUTION.

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IN 1901 Besredka[1] succeeded in getting filtrates of streptococcus cultures which were strongly hemolytic for red corpuscles of many animals. To get this hemolysin in solution he grew a highly virulent streptococcus in heated rabbit serum for 20 to 24 hours and passed the culture through a Chamberlain filter. He made a study of this substance and found that it does not pass through a dialysing membrane, that it deteriorates with age, and that it is destroyed by heating at 70° for two hours.

In the previous publication I[2] have verified and extended Besredka's observations. In that paper it was shown that streptolysin is destroyed by peptic digestion, that it is neutralized by a weak solution of formaldehyde, and that it behaves like a substance having a haptophore and a toxophore group firmly linked together. That is, at a temperature of 5° to 6° C. it attaches itself to the red corpuscles so firmly that it cannot be washed away by repeated washings of the corpuscles in cold salt solution. No hemolysis takes place as long as the temperature is kept below 6° C., but if the temperature of the suspension of washed treated corpuscles is raised to 30° to 40° C., the latter are rapidly hemolysed. It was shown further that heated chicken serum is quite strongly antihemolytic when added to streptolysin before the addition of the red corpuscles. If, however, the streptolysin is bound to the corpuscles in the cold, and the corpuscles are then washed and suspended in cold salt solution, the addition of chicken serum to this suspension does not prevent hemolysis when the temperature is brought up to 30° to 40° C. But if, instead of chicken serum, a trace of zinc chloride or zinc sulphate is added to such a suspension of washed treated corpuscles, no hemolysis takes place when the temperature is raised. That is, the chicken serum seems

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[1] *Ann. de l'Inst. Pasteur*, 1901, 15, p. 880.

[2] *Jour. Am. Med. Assoc.*, 1903, 41, p. 962.

to affect only the haptophore group, while the zinc salt ($ZnCl_2$) destroys or neutralizes the toxophore group after the streptolysin has been bound to the red corpuscles.

During the last three years a number of facts have been ascertained which throw additional light upon the nature of streptolysin.

Serum culture in an amount of 10 c.c. was evaporated to dryness by passing a current of dried air over it at room temperature. The residue was ground up in a mortar, further dried in a dessicator for 24 hours, and sterilized in dry heat at 150° C. for 30 minutes. The dried powder only partially dissolved in 0.85 per cent NaCl solution, but the clear centrifugated solution hemolysed rabbit corpuscles in two to three hours.

Attempts were made to get streptolysin in pure form—that is, to free it from the proteids of the serum. These attempts have not been wholly successful, but the results are interesting and worth recording. Twenty c.c. of filtered serum culture which was strongly hemolytic were saturated with magnesium sulphate at 35° C. and allowed to stand at this temperature for two hours. The precipitate was now filtered out, washed on the filter with a saturated solution of magnesium sulphate, and redissolved in 20 c.c. of 0.75 per cent NaCl solution. This solution and the filtrate were dialysed in separate dialysers for 24 hours against a continuous stream of distilled water. After dialysis enough NaCl was added to each lot to make a 0.75 per cent solution,[1] and each portion was tested for streptolysin with rabbit corpuscles. The test showed that the dissolved precipitate is strongly hemolytic, while the filtrate has no hemolytic powers; that is, the streptolysin is precipitated quantitatively with the globulin by saturation with $MgSO_4$ at 35° C. This result has also been obtained when the globulin was precipitated by mixing equal volumes of streptolysin (filtered serum culture) and a saturated solution of ammonium sulphate (saturated at 35° C.) and filtering after one to two hours. If the mixtures are filtered immediately after adding the salt, the filtrate contains some hemolysin, although the greater part of it is in the precipitate.

I now tried fractional salting-out of the globulin and tested each fraction for streptolysin. Euglobulin was precipitated by adding 5 c.c. of a saturated solution of $(NH_4)_2SO_4$ to 10 c.c. of a strongly hemolytic filtrate of a serum culture of streptococcus. After standing two hours the precipitate was collected on a filter and washed with a one-third saturated solution of $(NH_4)_2SO_4$ and dissolved in 0.75 per cent NaCl solution. The filtrate was now treated with 5 c.c. more of saturated $(NH_4)_2SO_4$ solution and the second precipitate (pseudoglobulin) collected on a filter, washed with a half-saturated solution of $(NH_4)_2SO_4$, and dissolved in 0.75 per cent NaCl solution. The solutions of euglobulin and of pseudoglobulin were each dialysed for 24 hours and (after adding enough NaCl to make a 0.75 per cent solution) tested for streptolysin with rabbit corpuscles. Both solutions were found to be quite strongly hemolytic.

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[1] A part of the globulin usually is precipitated and may not be completely redissolved in the salt solution.

Ten c.c. of streptolysin were now diluted with 90 c.c. of distilled water and a stream of $CO_2$ passed into the solution for one hour. The precipitate of insoluble globulin was centrifugated out of the liquid, washed twice with distilled water which had been saturated with $CO_2$, and then dissolved in 0.75 per cent NaCl solution. This solution was hemolytic for rabbit corpuscles, as was also the diluted streptolysin from which the insoluble globulin had been separated.

Very similar results were obtained by precipitation of the insoluble globulin by dialysis. Five c.c. of streptolysin were mixed with an equal volume of saturated solution of $(NH_4)_2SO_4$, and after standing two hours the precipitate was collected on a filter, washed with a half-saturated solution of $(NH_4)_2SO_4$, and dissolved in 0.75 per cent NaCl solution. This solution was dialysed against distilled water for 48 hours, at which time it contained a heavy precipitate. The precipitate was collected on a filter, washed three times with distilled water, and dissolved in 0.75 per cent NaCl solution. Not all of the precipitate went into solution, but the solution was found to be hemolytic for rabbit corpuscles. The filtrate containing the soluble globulin also had marked hemolytic powers.

Several attempts were made to extract the streptolysin from the filtered serum cultures by treatment with ether, alcohol, and chloroform, but all of these experiments gave negative results.

The normal serum of some animals, as for instance the chicken the goat, and the horse, has a decided antistreptolytic effect. This antistreptolytic action may be represented by a curve if falling quantities of the serum are added to tubes containing a definite quantity of streptolysin and washed corpuscles, and the percentages of hemolysis are taken as ordinates and the amounts of serum as abscissas. Fig. 1 shows such a curve obtained with heated goat serum as the antistreptolysin. Each tube contained 0.4 c.c. streptolysin, 0.2 c.c. of a 20 per cent suspension of washed rabbit corpuscles, falling quantities of heated goat serum, and enough 0.85 per cent NaCl solution to make 2 c.c. The tubes were incubated at 36° C. for two hours, during which time they were shaken once. At the end of the incubation they were centrifugated and the percentages of hemolysis determined with the aid of a calorimetric scale. If the percentage of hemolysis is carefully determined, the resultant curve is a fairly regular one.

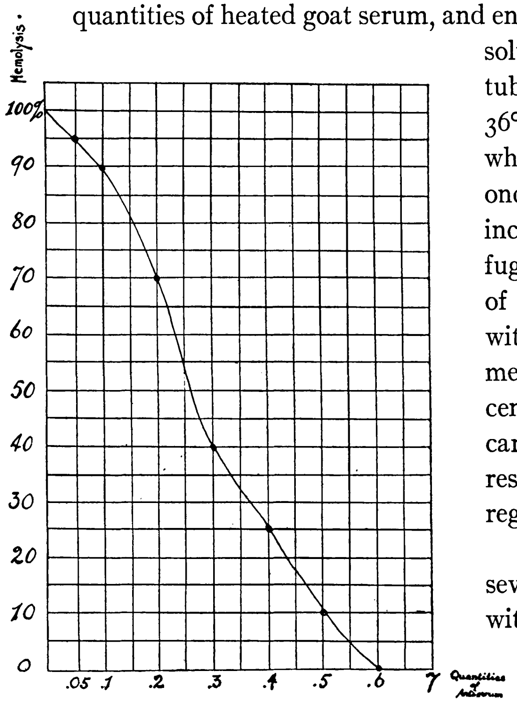
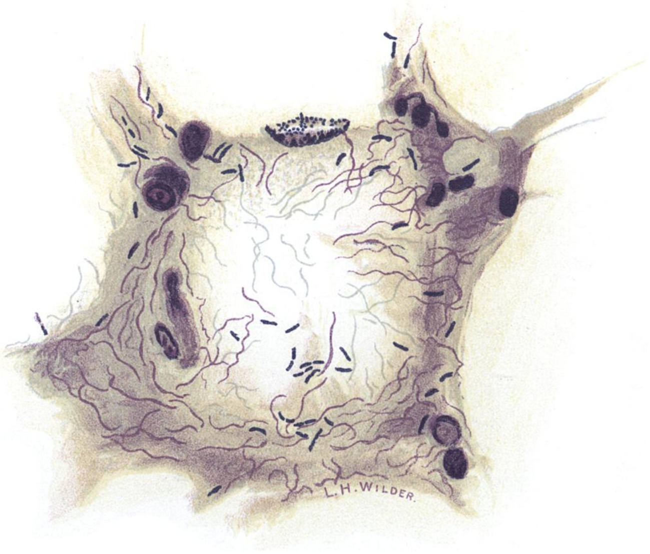


FIG. 1

A goat, a chicken, and several rabbits were injected with gradually increased quantities of streptolysin, but no specific antistreptolysin could be produced. The sera of several rabbits and of a horse which had been immunized against a virulent streptococcus were tested for antistreptolysin, but no definite increase in antistreptolytic power over that of normal sera could be detected. The sera of two rabbits seemed to have a slightly greater antistreptolytic action than the normal control, but the difference was so small that it may well have been due to a variation in the antistreptolytic power of the normal serum.

# PLATE 1*a*.



Section through the living subcutaneous tissue just beyond the advancing necrosis. About ×800.

Breton[1] and very recently Tchitchkine[2] claim to have been able to produce a specific antistreptolysin by immunizing rabbits with a virulent streptococcus. The antistreptolytic power of their sera was very little greater than that of the normal serum, and it seems likely, therefore, that they were not dealing with a specific antibody, but with the inhibitive action of normal serum. This view has already been expressed by Besredka[3] in regard to the antistreptolysin described by Breton.

### SUMMARY.

In summarizing we can say that streptolysin is a complex organic substance which has a haptophore and a toxophore group.

It is intimately associated with the globulins of the serum in which it is produced and has not yet been separated from them. It can be separated quantitatively from the serum albumin by saturation with magnesium sulphate at 35° C. or by half-saturation with ammonium sulphate, and it seems not unlikely that it is a globulin.

It is neutralized by a weak solution of formaldehyde and is destroyed by peptic digestion.

It cannot be extracted from the serum with alcohol, ether, or chloroform.

It does not pass through a dialysing membrane, but is destroyed by heating at 70° C. for two hours (Besredka).

Chicken serum, goat serum, and horse serum have marked antistreptolytic properties.

Attempts to produce a specific antistreptolysin have not been successful.

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[1] *Compt. rend. de la Soc. de biol.*, 1903, 55, p. 886.

[2] *Ann. de l'Inst. Pasteur*, 1906, 20, p. 506.

[3] *Ibid.*, 1903, 1, p. 575.